VOL. XII. NO. 6.—BOTANICAL GAZETTE.—JUNE, 1887.

The occurrence and function of certain nitrogenous bodies in plants.

W. E. STONE.

The nitrogenous bodies are probably the least understood of all plant constituents; indeed, the whole question of the relation of nitrogen to vegetation presents many unsolved problems. Its source, assimilation, various forms of existence, destruction, transformation and functions are all subjects of discussion at present, as they have been for many years. Something of the difficulty met with in the study of these questions arises from the chemical indifference of the element, and the difficulty of recognizing and isolating its various compounds as such. Micro-chemical methods fail for this purpose except in one or two cases, and, while purely chemical processes have accomplished a great deal, the discoveries announced from time to time, and the still existing gaps in theories, show that all the data are not yet at hand. A very practical and tangible illustration of this state of our knowledge occurs in the estimation and valuation of vegetable nitrogen as a food element. For lack of a better general knowledge and methods the total nitrogen present is regarded as existing in the form of albumin, or in a few cases is classified as albuminoid and non-albuminoid, although it is well known that it may be present in a variety of forms of very different value and structure. If these different nitrogenous bodies have different values to the animal system, it is quite certain that they represent different physiological offices and uses in the living vegetable organism.

During the past decade certain German investigators have done much toward determining (1) what these nitrogenous bodies are, and (2) what their functions may be. The results of their work, scattered through various chemical and botanical journals, are not generally available to American botanists, nor have they, so far as I know, been connectedly presented to them. For these reasons, no less than because it is a very important physiological subject, a general résumé has seemed desirable.

For the sake of convenience the vegetable nitrogenous bodies may be classified under two heads: those which play the part of reserve material, and those which are involved in the vital processes of the germinating or growing plant, or, passive and active forms. The work of the past few years has pointed out the existence of these classes and indicated their different values in the vegetable economy.

The reserve nitrogenous bodies occur chiefly in the form of albumin, or according to Ritthausen[1] *conglutin* and *legumin*, terms commonly applied to vegetable albumin or casein. These compose almost exclusively the nitrogenous constituents of seeds, but in roots and tubers are often accompanied by other bodies of a secondary and derivative nature, which may be regarded as surplus untransformed materials left by the cessation of the vegetative processes. The albumins, legumin and conglutin, which may be regarded as the true reserve forms of nitrogen, are colloids, are insoluble in acid fluids and, usually existing in a solid condition, are not directly available for the processes of transmutation or assimilation.

The active or secondary forms, occurring rarely in seeds but always present in germinating and, at least locally, in growing plants, we will discuss more in detail. They are chiefly amido compounds, crystalloids, and soluble in fluids of the plant. Because of their unstability and great solubility, their isolation is very difficult; for a long time their existence was not recognized, and even at present it may be affirmed safely that still others await discovery.

The most common of these, and, because of its comparatively easy recognition, the first which attracted attention, is asparagin,[2] now regarded as a generally disseminated plant constituent and proved to be present in a large number of families.[3] It was found in the young germs of *Lupinus luteus* by Beyer,[4] and subsequent study showed that the seeds and germs of this plant were peculiarly adapted to the investigation of these secondary nitrogenous compounds. The 10 to 12 per cent. of nitrogen in the seeds is almost entirely in the form of albumin, unaccompanied by asparagin, while the germs are unusually rich in asparagin and other non-albuminous nitrogenous bodies.

Pfeffer[5] made this the subject of especial study, and found

[1] Die Eiweiss Körper der Getreidearten, p. 188.

[2] $C_4H_8N_2O_3$. Discovered by Vauquelin and Robiquet in asparagus shoots, in 1805.

[3] For a list of plants in which it had been found at that date, see Die Pflanzenstoffe, Berlin, 1871, A. & T. Husemann.

[4] Landwirthschaft. Versuchs-Stationen, vol. 9 (1867), p. 168.

[5] Pringsheim's Jahrbuch für Botanik, vol. 8 (1872), p. 429.

that if the germs were excluded from light the amount of asparagin formed was greatly increased, particularly in the last stages of germination. He concluded that the asparagin was derived from the albumin originally occurring in the seed, and served for the transportation of the same to the growing parts of the plant—a process analogous to the solution and transmutation of insoluble carbohydrates. His deductions were confined, however, to the Leguminosæ, outside of which he does not mention the occurrence of asparagin.

In 1876 E. Schulze[6] published the first of a notable series of contributions upon the subject. Lupine seeds which contained 45 per cent. of albumin were germinated and grown fifteen days under exclusion of light. At the end of this time the total amount of nitrogen present was unchanged, but only 8 per cent. of albumin remained; 37 per cent. had been changed to a soluble form, of which nearly two-thirds was asparagin. By repeated experiments it was found that the most favorable conditions for the formation and accumulation of asparagin were germination in the dark for ten days, and then exposure to faint light for some weeks[7]; the germs thus produced contained usually 27 to 28 per cent. of their dry weight in asparagin. The quantity produced was proportional to the time of growth, and it was found in the greatest abundance in the axial organs. These observations were supplemented by those of Borodin,[8] who gave to the question a new aspect by investigating the different parts of growing plants.

In the young shoots of many trees and shrubs he found appreciable quantities of asparagin; in some, however, only traces; while in others, as Larix, Betula, Alnus, Syringa, Lonicera, etc., none at all could be recognized. But by modifying the conditions, viz., by detaching the twigs and allowing the buds to develop in water at ordinary room temperature, he was able to produce asparagin in all of the many species examined. He concluded, with Pfeffer and Schulze, that it could only come from a breaking down of albumin, and that it probably served as a source for the regeneration of the latter at a later stage.

The study of the problem thus far had been prosecuted

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[6] Berichte d. Deutschen Chem. Gesellschaft, vol. 9 (1876), p. 1314. Also Landwirthschaft. Jahrbücher, vol 5 (1876), p. 821.

[7] Journal für Practische Chemie, vol. 27 (1883), p. 337.

[8] Botanische Zeitung, vol. 36 (1878), p. 801.

chiefly by micro-chemical methods, with the result of establishing the general occurrence of asparagin, and the theory that it served as a transferring agent between reserve albumin and the vegetative organs of the plant. This theory lacked general application because in some cases asparagin could not be detected and in others occured in too small quantities to satisfy the requirements of the plant if the theory was true. Either the hypothesis was not correct or there must be other products of the breaking up of albumin which the methods thus far used could not detect.

The identification of such products by chemical methods not only supplied facts of the most vital importance to the question under consideration, but serves to illustrate the dependence of vegetable physiology upon the chemist as well as the microscopist for the solution of its problems.

Shortly before Borodin's investigations Schulze had taken up the study of the seeds and young germs of Cucurbita Pepo.[9] Pfeffer had not found asparagin here, but Sabanin and Laskowsky[10] had obtained secondary ammonia salts in watery extracts from them, without, however, determining their nature. Schulze, by a peculiar method of treatment, was able to isolate glutaminic acid, which was assumed to exist originally in the juices of the germ as an amide.[11] This amounted to 1.75 per cent. of the dried weight of the germs, which were grown sixteen days under exclusion of light. As no such substance occurred in the ungerminated seeds he concluded that like the asparagin of the lupine it was derived from the breaking down of albumin, and performed a similar office to the plant. Later[12] traces of asparagin were found together with small quantities of tyrosin and leucin,[13] all secondary, soluble, nitrogenous bodies, to which were subsequently added vernin,[14] xanthin bodies, ammonia salts and nitrates.[15]

Meanwhile the lupine which had produced asparagin so freely, had also been examined for other nitrogenous bodies, with rich results.[16] Beside the small quantities of glutamin, leucin, tyrosin, and possibly ammonia salts, which it pro-

[9] Berichte d. Deutschen Chemischen Gesellschaft, vol. 10 (1877), p. 199.
[10] Landwirthschaft. Versuchs-Stationen, vol. 18, p. 405.
[11] Probably as $C_5H_6(NH_2)_2O_3$.
[12] Journal für Practische Chemie, vol. 20, p. 385.
[13] Tyrosin=$C_9H_9(NH_2)O_3$. Leucin=$C_6H_{11}(NH_2)O_2$.
[14] Vernin=$C_{16}H_{20}N_2O_2$. See Zeitschrift für Phisiolog. Chem., vol. 10, p. 80.
[15] Journal für Practische Chemie, vol. 32, p. 433.
[16] Journal für Practische Chemie, vol. 27, p. 337.

duced in common with Cucurbita, two *amido* acids[17] and peptone were found; while during the last year it has yielded still another new body, arginin.[18]

These substances are all regarded as secondary products derived from the albumin of the seed in a manner analogous to the formation of asparagin. Confirmatory of this view is the fact that the albumin of lupine seeds when subjected to artificial chemical action is converted into amido acids which seem to be identical with those produced in germination.[19]

It was noteworthy that the amido acids which were so distinctly present in the germs of lupine were not found in those of Cucurbita, and that the asparagin of the former was substituted by glutamin in the latter.

Following Borodin's study of the occurrence of asparagin in the young shoots of woody plants, Schulze conceived that other bodies might be found here as well as in the germinating stages already examined. Twigs of Platanus occidentalis were removed from the tree in April and kept in a warm room until no further growth was made. The young shoots thus developed contained, besides asparagin, an appreciable quantity (.5 to 1 per cent.) of a substance corresponding in its reactions to allantoin.[20] Repeated investigations showed this to be a constant constituent of young shoots of Platanus treated in this way, but it could not be detected in shoots or leaves growing normally upon the tree. Leucin and bodies of the xanthin group were also found in Platanus. Allantoin also occurs in the shoots of Acer pseudo-platanus.

The vernin, already mentioned in connection with lupine, seems also to be widely disseminated, occurring in the germs of Vicia sativa (in which it was discovered), Trifolium pratense, Cucurbita Pepo, the sclerotium of Claviceps purpurea, and the pollen of Pinus sylvestris and Corylus avellana.[21]

The examples given show that in the germinating and vegetative stages of the plant, secondary nitrogenous bodies appear in considerable variety of form, and in sufficient quantities to imply some important use or office. The idea that nitrogenous, and particularly albuminous, substances undergo changes during growth and assimilation is by no means

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[17] Phenyl-amido-propionic acid=$C_9H_9(NH_2)O_2$. Amido-valerianic acid $C_5H_9(NH_2)O_2$

[18] $C_6H_{14}N_4O_2,HNO_3+\frac{1}{2}H_2O$. See Berichte d. Deutsch. Chem. Gesellsch., vol. 19 (1886), p. 1177.

[19] Schulze Zeitschrift für Physiol. Chem., vol. 9, p. 63; same vol., p. 253: vol. 10, p. 134; vol. 11, p. 210. Also Gorup-Besanez, Berichte d. Deutsch. Chem. Gesellsch., vol. 10, p. 780.

[20] $C_4H_6N_4O_3$. Berichte d. Deutschen. Chem. Gesellschaft, vol. 14 (1881), p. 1602.

[21] A. von Planta. Landwirthschaft, Versuchs-Stationen, vol. 31, p. 97, and vol. 32, p. 215.

new. Liebig regarded these bodies as possessing the nature of ferments,[22] but Hartig first indicated the probable breaking up of reserve albumin into crystallenic compounds.[23] Pfeffer in his thorough discussion of the appearance of asparagin, already referred to, ascribed its origin to the original albumin present, and pointed out that in the change C and H were set free, either to be exhaled or adapted to the building of new tissue.[24]

The exclusion of light favored its formation, but he afterwards found that the process followed equally well in the light if CO_2 was excluded. This led him to conclude that the formation of asparagin was in some way connected with the presence or absence of carbohydrates in the plant. Borodin found it accumulating in young shoots and buds not alone when detached from the parent stem, but equally as well when, remaining in position, they were protected from light. He also concluded that the phenomenon was caused by the lack of non-nitrogenous substances, based upon one of two principles, either, first, albumin undergoes no breaking down in the presence of carbohydrates; or, second, it constantly undergoes transformation, but in the presence of carbohydrates is constantly regenerated to new albumin, and no accumulation of the secondary products takes place.[25]

The latter supposition seemed the more probable, since the asparagin which collected in etiolated growths disappeared again when they were exposed to light, and albumin increased proportionally.

Schulze's discovery of so many other bodies homologous to asparagin, served to strengthen and broaden the application of the theory of the transformation of albumin. He showed farther that in the germs where the secondary products accumulated, there was an increase of H_2SO_4 corresponding to the amount of S set free from the broken down albumin molecules,[26] and Pfeffer regards the exhaled CO_2 as also coming from this source.[27]

The dependence of these bodies upon the absence of nitrogen-free substances for their formation seems also clearly indicated by the observations upon etiolated growths, and

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[22] Die Organische Chemie in ihrer Anwendung auf Agricultur, 1840, p. 220.

[23] Entwickelungs-Geschichte der Pflanzen-Keim, 1858, p. 126.

[24] Botanische Zeitung, vol. 8 (1872), p. 429. See also Physiological Botany, Goodale, p. 365.

[25] Botanische Zeitung, vol. 36 (1878), p. 801.

[26] Berichte d. Deutschen Chem. Gesellschaft, vol. 9 (1876), p. 1314; vol. 11 (1878), p. 438; vol. 13 (1880), p. 21.

[27] Handbuch der Pflanzenphysiologie, p. 295.

the effect of withholding CO_2. Farther Schulze has shown[28] that those seeds containing proportionally large amounts of albumin and small quantities of carbohydrates, produce amide compounds most abundantly in germination. The ratios of albumin to nitrogen-free substances in seeds of lupine and Cucurbita were respectively 1 : 0.35 and 1 : 1.56. After fifteen days' germination, the lupine produced 19.43 per cent. of the dry weight of the seeds in asparagin and glutamin, and Cucurbita only 5.78 per cent.

The office of the vegetable amides is, therefore, as at present understood, to serve as a transferable form of nitrogen from reserve supplies to the place of growth, and farther, probably, as an indirect source of non-nitrogenous materials. Schulze has shown that they may also act as reserve material, in certain cases, in roots and tubers.[29] They occur universally in the germinating and vegetating stages of the plant, but fail almost as invariably in seeds. By their formation, through the breaking up of the albumin molecule, C and H are liberated, which may assist in the formation of new tissues, especially when assimilation has been artificially prevented or before it has begun in the germ. A like checking of assimilation in growing shoots, or a cutting off of the supply of nitrogen-free materials from the main stem, also results in an accumulation of these bodies. The small quantity of amides, formed by normal germination, is regenerated to albumin as soon as assimilation begins, while in the normal growth of plants they are found only sparingly, and in some cases not at all, or at least are not detected by our present methods. So much has been determined with reasonable certainty in our knowledge of these substances.

There remains the question whether the amides serve ordinarily for the transfer of nitrogen alone, while their ability to assist in the formation of carbohydrates is exerted only at stated periods or in emergencies, or whether these two processes are supplementary and constantly operating.

Pfeffer advances two hypotheses.[30] Either in every cell of growing tissue a transformation of albumin to amides is constantly occurring, and under normal conditions an equally constant regeneration to albumin follows, or such transformation occurs first when other nitrogen-free materials are

[28] Landwirthschaft-Jahrbücher, vol. 9 (1880), p. 733.
[29] Berichte d. Deutsch. Chem. Gesellschaft, vol. 16 (1883), p. 312; vol. 18 (1885), p. 390. Landwirth. Versuchs-Stationen, vol. 29, p. 295.
[30] Handbuch der Pflanzenphysiologie, p. 299.

lacking. Both may be true, and he points out the analogy of fungi which are able to make growth, forming cellulose and oils, with a nourishment of only nitrogenous substances, but if carbohydrates be also furnished them, a much smaller quantity of the nitrogenous matter will be made use of.

The principal amides observed under normal conditions, are, as already stated, asparagin and glutamin. Schulze assumes[31] that this does not indicate a larger production of these particular bodies, but that they are less easily regenerated than some other forms. In this way he explains the fact that the amount of asparagin in lupine germs continued to increase for some time after their exposure to light, at the same time assuming that the first carbohydrates formed by renewed assimilation are employed for the formation of new tissues, and not for the formation of albumin from amides.

The observations of Borodin and Schulze also indicate that not all carbohydrates or nitrogen-free substances have the properties for influencing the formation of amides or their regeneration. For instance, sugar beets contain considerable quantities of amides and cane sugar, existing side by side, and the conclusion is that cane sugar is unable to promote the regeneration of the amides or participate in the formation of new tissues. Fungi invert cane sugar before transmutation, and a similar process may be necessary before the regeneration of amides is possible. Probably only a few of the nitrogen-free bodies have this property; perhaps only glucose.

The question as to why the presence of nitrogen-free bodies is necessary to the regeneration of the amides also presents some difficulties. Since asparagin and glutamin contain less C and H than albumin, an addition of these elements is necessary to the formation of the latter. But tyrosin and leucin contain comparatively more C and H than albumin. Judging from their elementary composition, therefore, if one group requires the presence of carbohydrates for regeneration, the other does not. It is possible that direct combinaton between the two groups occurs with the co-operation of carbohydrates. At least Schulze's conclusion, that the carbohydrates *promote* the reconstruction of albumin from the secondary nitrogenous compounds, seems safely acceptable in the light of our present knowledge.

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[31] Landwirthschaft. Jahrbüch, vol. 9 (1880), p. 731.